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Hartmann, A A ; Buchmann, N ; Niklaus, P A

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# A study of soil methane sink regulation in two grasslands exposed to drought and N fertilization

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**Abstract** Oxidation by soil bacteria is the only biological sink for atmospheric methane ( $\text{CH}_4$ ). There are substantial uncertainties regarding the global size of this sink, in part because the ecological controls of the involved processes are not well understood to date. We have investigated effects of severe summer drought and of nitrogen inputs (ammonium nitrate or cattle urine) on soil  $\text{CH}_4$  fluxes in a field experiment. Soil moisture was the most important factor regulating the temporal dynamics of  $\text{CH}_4$  fluxes. Simulated drought episodes altered the soil's water balance throughout the year, increasing  $\text{CH}_4$  oxidation by 50% on an annual basis. N fertilizers exerted only small and transient effects at the ecosystem level.

Laboratory incubations suggested that effects differed between soil layers, with larger effects of drought and N application in the top soil than in deeper layers. With soil moisture being the primary controlling factor of methanotrophy, a detailed understanding of the ecosystem's water balance is required to predict  $\text{CH}_4$  budgets under future climatic conditions.

**Keywords** Ammonium nitrate · Cattle urine · Drought · Enzymatic inhibition · Grazing

## Introduction

Methane ( $\text{CH}_4$ ) is the second-most important anthropogenic greenhouse gas after carbon dioxide ( $\text{CO}_2$ ), despite its atmospheric mixing ratio of only  $1.8 \mu\text{L L}^{-1}$  (IPCC 2007). While many  $\text{CH}_4$  sources exist, both natural and anthropogenic, there is just one relevant biological sink for  $\text{CH}_4$  – the oxidation of  $\text{CH}_4$  in soils by methanotrophic bacteria (Dunfield 2007). Methanotrophs in wetland soils mainly thrive on  $\text{CH}_4$  produced by methanogenesis in deeper soil layers where redox potential is low (Le Mer and Roger 2001). In contrast, well-aerated upland soils generally act as a net sink for atmospheric  $\text{CH}_4$  (Conrad 2009). Global soil  $\text{CH}_4$  sink estimates average around  $30 \text{ Tg yr}^{-1}$  (IPCC 2007), but this number is associated with large uncertainties (Smith et al. 2000), in part because the environmental

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regulation of the  $\text{CH}_4$  uptake in upland soils is only poorly understood to date (Dunfield 2007; Bodelier and Laanbroek 2004).

Soil  $\text{CH}_4$  uptake is generally limited by diffusion of  $\text{CH}_4$  and dioxygen ( $\text{O}_2$ ) through the soil profile to the sites occupied by  $\text{CH}_4$  oxidizing bacteria (Del Grosso et al. 2000). The main factors determining soils gas diffusivity are soil porosity and water content. Soil moisture limits  $\text{CH}_4$  and  $\text{O}_2$  diffusion by filling small pore networks and, at high soil moisture, also macro-pores. Diffusion of  $\text{CH}_4$  in water is slow and solubility low, so that soil moisture very effectively blocks  $\text{CH}_4$  transport in soil. On the other hand, extreme water deficiency can reduce soil  $\text{CH}_4$  uptake due to physiological stress (Nesbit and Breitenbeck 1992).

A number of studies have shown that fertilizers containing ammonium ( $\text{NH}_4^+$ ) can reduce soil  $\text{CH}_4$  oxidation in a variety of ecosystems (Bronson and Mosier 1994; Hütsch et al. 1994; Powlson et al. 1997). However, the mechanisms underlying this effect remain unclear. While  $\text{NH}_3$  can inhibit the enzyme system responsible for the oxidation of  $\text{CH}_4$  in methanotrophs (Dunfield and Knowles 1995), reports are inconsistent. Many experiments have shown patterns consistent with the hypothesis of competitive inhibition at the enzyme level (Mosier et al. 1991; Bronson and Mosier 1994; Willison et al. 1995). However, inhibitory effects occurred only with a delay in other studies (Hütsch et al. 1993; Gulledge et al. 1997). Still other experiments showed no effects of fertilizer application (Lessard et al. 1997), or even a positive effect of N application (Bodelier et al. 2000), indicating that the effect of N fertilization on  $\text{CH}_4$  uptake of soils is more complex (Bodelier and Laanbroek 2004). These findings probably reflect the fact that  $\text{NH}_4^+$ , while inhibiting  $\text{CH}_4$  assimilation, also is an essential nutrient for methanotrophs. It has also been proposed that reductions in  $\text{CH}_4$  oxidation rates may result from general osmotic effects of the applied fertilizer (e.g. Gulledge and Schimel 1998).

Grazing animals redistribute ingested plant N in patchy form, resulting in local N deposition rates of several hundred or thousand  $\text{kg N ha}^{-1}$  under dung and urine patches (Haynes and Williams 1993). Many pasture soils oxidize atmospheric  $\text{CH}_4$  (Smith et al. 2000), despite these very high patch-level N deposition rates. This contrasts the strong and persistent reductions in soil  $\text{CH}_4$  uptake often found under

agricultural fertilizer application (e.g. Hütsch et al. 1993). Is methanotrophy under excreta patches inhibited only temporarily, or are methanotrophs eliminated for longer time periods? Were methanotroph species in grazed ecosystems selected to tolerate these extreme conditions?

In the present study, we investigated soil  $\text{CH}_4$  uptake in two multi-year field experiments in temperate grassland. N fertilizer was applied at rates equivalent to local N deposition by cattle, and severe summer drought was simulated with rain exclusion roofs. The objectives of this study were to analyze the mechanisms by which these experimental treatments affect soil  $\text{CH}_4$  uptake, and to test how these interact.

## Materials and methods

### Field sites and experimental design

A field experiment simulating drought and N deposition by cattle was set up in September 2006 on two research farms representing typical Swiss grassland farming systems. The first site, *Früebüel* (Fig. 5 of online resource 1), is located on a montane plateau in central Switzerland and managed at intermediate intensity (8.5415° E, 47.1135° N, 1,000 m a.s.l.). Prior to this study, field plots were grazed by non-dairy cattle or mown for hay three to four times per year. The only fertilizer inputs were cattle excreta and manure from cattle kept in stables. The growing period lasts from April to October. The soil is a silt loam (37% sand, 56% silt and 7% clay, pH of ~4.7). The second site, *Alp Weissenstein* (Fig. 6 of online resource 1), is an extensively managed subalpine grassland in the eastern Swiss Alps (46.5833° E, 9.7859° N, 1,975 m a.s.l.). The growing period is short (mid May to mid September) and the site only grazed two to three times by non-dairy cattle and horses. No fertilizer is applied. The soil is a silt loam (35% sand, 59% silt and 6% clay) with a pH of ~5.0. Mean annual temperature and precipitation for the 1961–1990 period are approximately 6–7°C and 950 mm for Früebüel and 2°C and 1,350 mm for Alp Weissenstein (data provided by Swiss Federal Office of Meteorology and Climatology, values interpolated from nearby weather stations correcting for altitude).

The field experiment was organized as randomized complete split-plot design with five blocks per site.

Each block consisted of two 3.5 m×3 m plots, one of which was subject to simulated drought while the other served as control plot. Drought was simulated by excluding precipitation with rain exclusion roofs (Gilgen and Buchmann 2009) covered with a 200 µm thin plastic foil (Gewächshausfolie UV 5, folitec Agrarfolien-Vertriebs GmbH, Westerbürg, Germany). The central 2 m×2.2 m of each plot were subdivided into four subplots using polyvinyl chloride sheets reaching 15 cm depth. These subplots were either treated with ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>), non-dairy cattle urine, or served as unfertilized control (NIL). The forth subplot was not used in this study. This setup resulted in five replicates per site and treatment combination.

Ten-minute averages of soil temperature and moisture were recorded in two blocks per site (CR1000 data logger, Campbell Scientific Inc., Logan, UT, USA) at two depths (8 and 25 cm at *Früebüel* and 8 and 20 cm in the shallower soils at *Alp Weissenstein*). Temperature probes (AD592, Analog Devices, Norwood, MA, USA) were installed in all subplots, whereas soil moisture probes (EC-6, Decagon Devices Inc., Pullman, WA, USA) were installed in the unfertilized subplots only. Concomitantly with the regular CH<sub>4</sub> flux measurements, soil moisture was recorded manually in all plots (0–5 cm depth, ThetaProbe ML2x, Delta-T Devices Ltd., Burwell, Cambridge, UK).

## Management

Grazing animals were excluded from October 2006 until the experiment was terminated in summer 2009. The sites were clipped at 4 cm height when the surrounding pastures were grazed by livestock or mown by the local farmer.

In 2007, the rain-exclusion roofs were installed from August 3 to September 27 and from July 31 to September 25 at *Früebüel* and *Alp Weissenstein*, respectively. In 2008, the roofs were installed from June 26 to August 13 and from July 14 to September 26, respectively.

The fertilizer treatment consisted of a small and a larger application in 2007 and a single large application in 2008. All applications took place when the rain exclusion roofs were installed. We split the fertilizer application in the first year into two portions because we had no a priori knowledge of the

sensitivity of the soil CH<sub>4</sub> sink and did not want to completely inhibit it over prolonged periods. Urine was collected from non-dairy cattle. Both NH<sub>4</sub>NO<sub>3</sub> and urine were applied as aqueous formulation (4.9 L m<sup>-2</sup>) and the same amount of water was applied to unfertilized control subplots (NIL treatment). The vast majority of urine N is in the form of urea, which quickly hydrolyses to NH<sub>4</sub><sup>+</sup> in soils (Haynes and Williams 1992). Since NH<sub>3</sub> is the chemical species generally believed to inhibit CH<sub>4</sub> oxidation, equivalent amounts of NH<sub>4</sub><sup>+</sup> and urea N were applied to subplots (5 g and 15 g N m<sup>-2</sup> for the small and large fertilizer applications, respectively), which resulted in double the amount of N applied in the NH<sub>4</sub>NO<sub>3</sub> fertilizer relative to the urine treatment.

## CH<sub>4</sub> flux measurements

Static chambers (32 cm diameter×30 cm height) with a detachable lid were installed in the center of all subplots by carefully pre-trenching the soil with a spade fitting the curvature of the chamber and lowering these 19 cm into the ground (resulting in 11 cm chamber height and 8.85 L headspace). Soil CH<sub>4</sub> uptake rates were measured approx. every 2 weeks during the growing period, and more frequently in the first week after fertilizer applications. To measure CH<sub>4</sub> exchange rates, lids were attached to the chamber collars and headspace samples collected 5, 20 and 35 min after chamber closure. Gas samples were injected into pre-evacuated exetainers and analyzed for CH<sub>4</sub> in the laboratory (Agilent 6890 gas chromatograph equipped with a flame ionization detector, Agilent Technologies Inc., Santa Clara, CA, USA). The small concentration changes (generally around ~0.2 µL L<sup>-1</sup>) did not allow to distinguish between zeroth or first order kinetics, and we therefore estimated CH<sub>4</sub> flux rates by linear regression. Correlation coefficients were generally very high ( $r^2 > 0.97$ ) unless CH<sub>4</sub> exchange rates were close to zero.

CH<sub>4</sub> concentrations were measured in soil air collected from 50 cm long polypropylene tubes (Accurel PP V8/2 HF, Membrana GmbH, Wuppertal, Germany) installed horizontally at the same depths as the soil moisture and temperature probes. These tubes are permeable for gases including CH<sub>4</sub> but not for water. They were closed at the ends to equilibrate with the soil atmosphere. On sampling dates, equilibrated air was collected with a syringe and CH<sub>4</sub> concentrations analyzed as described above.

## Soil analysis

When the experiment was terminated in 2009, soil blocks with 20 cm×20 cm surface area were excavated and divided into 0–5, 5–10 and 10–15 cm depth layers. CH<sub>4</sub> oxidation under standardized conditions was determined in fresh sieved soil (4 mm mesh size) corresponding to 100 g dry weight. This soil was adjusted to a water content of 0.3 g H<sub>2</sub>O (g soil)<sup>−1</sup> and placed into 0.9 L gas-tight jars fitted with a septum. The soils were equilibrated at 20°C overnight, the jars opened for 30 min to aerate the samples, closed again, and CH<sub>4</sub> oxidation rates determined by measuring headspace CH<sub>4</sub> concentrations after 10, 160 and 310 min. Incubations were conducted under atmospheric concentrations, i.e. no extra CH<sub>4</sub> was injected into the headspace.

A separate incubation experiment tested effects of chemical species on soil CH<sub>4</sub> oxidation. The underlying rationale was that inhibitory effects could be due to osmotic effects rather than to effects of the N contained in the fertilizers. Sieved top soil (0–10 cm) samples equivalent to 100 g dry weight were amended with 8 mL aqueous solution containing 0.0, 0.088, 0.175, 0.35, 0.70 or 1.40 mmol of NH<sub>4</sub>NO<sub>3</sub>, ammonium chloride (NH<sub>4</sub>Cl), potassium nitrate (KNO<sub>3</sub>) or potassium chloride (KCl). Soils were incubated overnight in 0.9 L jars from which septa had been removed and blocked with paper tissue to allow some air exchange without drying of soils. Then, jars were aerated for 30 min and CH<sub>4</sub> oxidation rates measured as described above. The measurements of CH<sub>4</sub> consumption were repeated daily to test for a temporal component of effects. However, because there was none, these measurements were discontinued after 4 days and repeated measures averaged.

## Data analysis

Data were analyzed using mixed-effects models fitted by maximum likelihood (*lme* function from the *nlme*-package of R 2.8.1, R Development Core Team 2010). The models included the nested random effects site, block, plot, and subplot while drought, fertilization, and, where appropriate, also soil layer, were the fixed effects tested. Effects were considered significant when  $P < 0.05$ . All error estimates in text and figures are standard errors of treatment means.

The response of CH<sub>4</sub> oxidation to salt additions was described as sigmoidal function with intercept:

$$f_{\text{CH}_4} = x_1 \cdot (1 - 1/(1 + \exp(-x_2 \cdot (\text{conc} - x_3)))) + x_4,$$

where  $f_{\text{CH}_4}$  is the measured flux rate, conc is the concentration of the added compounds, and  $x_{1...4}$  are shape parameters estimated by non-linear least squares fitting using the *optim* function of R.

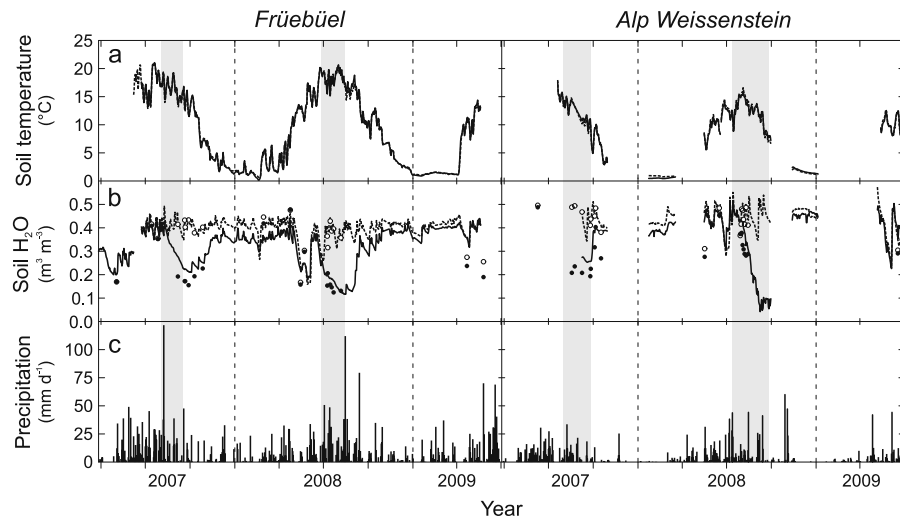
## Results

### Soil temperature and moisture

Soil temperature did not depend on simulated drought or N fertilization (Fig. 1a). Soil moisture exhibited large intra-annual variation with pronounced drying cycles (Fig. 1b). The rain exclusion roofs reduced precipitation in drought-treated plots in *Früebüel* by 450 mm and 410 mm in 2007 and 2008, respectively. The corresponding reductions at *Alp Weissenstein* were 210 mm and 315 mm. These effects are equivalent to a reduction in annual precipitation of 25–30%, or of 30–40% on a vegetation period basis (Fig. 1c). Soil moisture in drought-treated plots was reduced by 40–60% when rain exclusion roofs were installed (Fig. 1b;  $P < 0.001$ ). The reduction in soil moisture persisted for weeks or even months after the rain exclusion roofs had been removed. Most remarkably, significantly reduced soil water contents were repeatedly found up to 1 year after the roofs had been removed (i.e. in spring and summer of 2008 and 2009).

### Soil CH<sub>4</sub> exchange

CH<sub>4</sub> uptake rates were lower at *Früebüel* than at *Alp Weissenstein* (Fig. 2). Soil CH<sub>4</sub> uptake was strongly correlated with soil moisture (Fig. 2,  $r^2 = 0.67$  for *Früebüel* and  $r^2 = 0.80$  for *Alp Weissenstein*, respectively;  $P < 0.001$  for both sites), while soil temperature did not explain any additional variation when fitted after soil moisture in a multiple regression model. Rain exclusion roofs increased the soil CH<sub>4</sub> sink by over 100% at *Früebüel* and by over 200% at *Alp Weissenstein* when installed (Fig. 3a,  $P < 0.001$ ). After removal of the rain exclusion roofs, soil CH<sub>4</sub> uptake rates remained enhanced for several weeks while soil moisture was still reduced. Most interestingly, increased CH<sub>4</sub> sink rates were also found before the rain exclusion roofs were set up again in 2008 and 2009, i.e. up to 1 year after the rain exclusion roofs had been removed from the plots the year before.



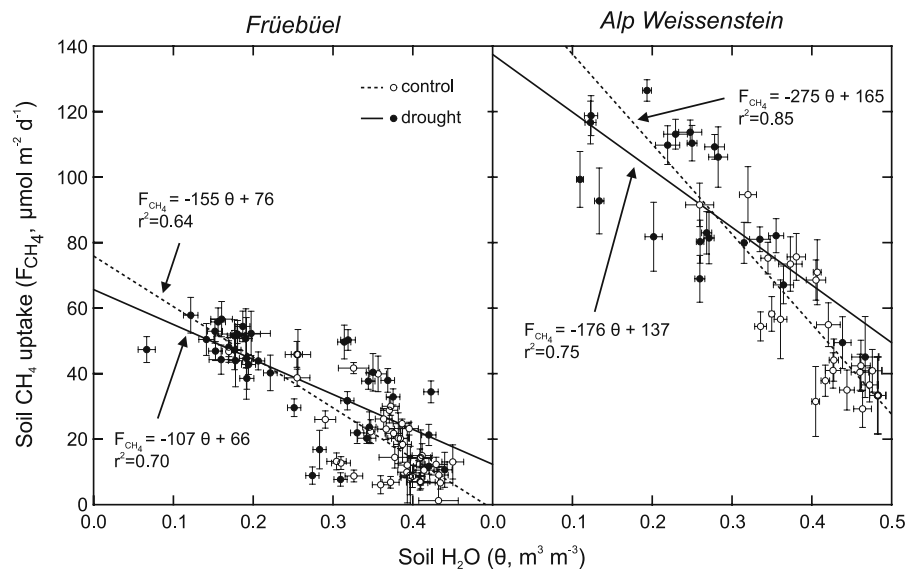
**Fig. 1** **a** Soil temperature at 8 cm soil depth for control (dashed line) and drought-treated plots (solid line); differences are not visible most of the time since there were no significant differences. **b** Soil moisture at 8 cm depth for control (dashed line) and drought treated plots (solid line). Additional manual

soil moisture measurements in 0–5 cm soil depth are indicated by dots (white circle control plots; black circle drought-treated plots). **c** Precipitation (data by Zeeman et al. 2010). Grey areas indicate periods with installed rain exclusion roofs

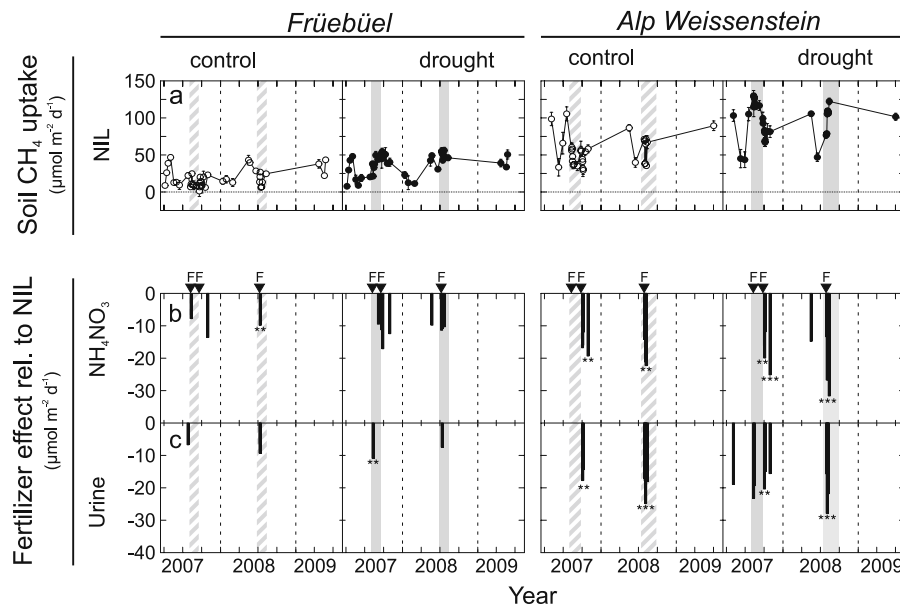
Both N fertilizers inhibited soil  $\text{CH}_4$  uptake at both sites, but effects were limited to a period of a few weeks after fertilizer application and then vanished. Averaged over a 30-day period after N application, soil  $\text{CH}_4$  uptake was reduced by both fertilizers ( $P < 0.05$ , average reduction of 13% over both sites). These inhibitory effects were relatively larger at

Fruebüel than at Alp Weissenstein ( $P < 0.05$  for site  $\times$  N). At Fruebüel, soil  $\text{CH}_4$  uptake was repeatedly found to be more strongly inhibited by N application in the drought treatment than in the control plots ( $P < 0.05$  for N  $\times$  drought). However, these effects were transient and occurred only on the first day after fertilizer applications or shortly thereafter.

**Fig. 2** Linear regression between soil  $\text{CH}_4$  uptake and soil moisture for both experimental sites. Measurements were conducted between spring 2007 and summer 2009. Error bars indicate standard errors







**Fig. 3** Soil CH<sub>4</sub> uptake at the two experimental sites Fruebuel and Alp Weissenstein. **a** shows CH<sub>4</sub> exchange rates in the unfertilised subplots (NIL treatment). **b** and **c** show effects of the added fertilizers relative to the NIL treatment; the presence of a bar indicates a significant effect at  $P < 0.05$ ; higher

significances are indicated as \*\*  $P < 0.01$  and \*\*\*  $P < 0.001$ ; for clusters of bars, only the highest significance is indicated. Grey areas indicate periods when rain exclusion roofs were installed; F indicate fertilizer applications; error bars indicate the standard errors of treatment means

### Soil CH<sub>4</sub> concentrations

Soil CH<sub>4</sub> concentrations always were below atmospheric concentration and decreased with depth (Table 1,  $P < 0.001$ ), indicating that both sites were a net sink for atmospheric CH<sub>4</sub>. Rain exclusion roofs increased soil CH<sub>4</sub> concentrations when installed (+20% at Fruebuel,  $P < 0.05$ , and +70% at Alp Weissenstein,  $P < 0.001$ , average over 2007 and 2008). This increase persisted for several weeks after removal of the rain exclusion roofs and closely followed the observed effects on soil moisture (data in online resource 2). As for soil moisture, a drought effect was detected in spring and summer of the next

year (before rain exclusion roofs were re-installed) with increased soil CH<sub>4</sub> concentrations occurring mainly at 8 cm soil depth (data in online resource 2).

The application of NH<sub>4</sub>NO<sub>3</sub> and urine had no significant effect on soil CH<sub>4</sub> concentrations, although soils at Alp Weissenstein exhibited a non-significant tendency towards increased soil CH<sub>4</sub> concentrations immediately after fertilizer application.

### CH<sub>4</sub> oxidation of sieved soil

CH<sub>4</sub> oxidation rates of sieved soils exposed to standardized soil moisture and temperature depended on site and soil depth (Table 2,  $P < 0.001$  for site and

**Table 1** Average soil CH<sub>4</sub> concentrations (μL L<sup>-1</sup>) measured in two soil depths during the periods in which rain exclusion roofs were installed in 2007 and 2008

Treatment	Fruebuel		Alp Weissenstein	
	8 cm	25 cm	8 cm	20 cm
Control	1.32±0.01	0.80±0.02	0.74±0.01	0.26±0.00
Drought	1.50±0.00	1.02±0.02	1.19±0.01	0.56±0.02

**Table 2** CH<sub>4</sub> oxidation (nmol CH<sub>4</sub> (g dry soil)<sup>-1</sup> d<sup>-1</sup>) of sieved soil exposed to ambient CH<sub>4</sub> concentrations, 20°C and with soil moisture adjusted to 0.3 m<sup>3</sup> H<sub>2</sub>O m<sup>-3</sup>

Treatment		Früebüel			Alp Weissenstein			average by treatment
		0–5 cm	5–10 cm	10–15 cm	0–5 cm	5–10 cm	10–15 cm	
Control	NIL	0.43±0.04	0.45±0.04	0.39±0.06	2.18±0.21	1.93±0.16	1.24±0.12	1.10±0.14
	NH <sub>4</sub> NO <sub>3</sub>	0.33±0.09	0.44±0.07	0.33±0.06	1.83±0.25	2.06±0.26	1.44±0.31	1.07±0.15
	Urine	0.38±0.04	0.40±0.03	0.37±0.04	1.83±0.21	1.54±0.16	1.11±0.08	0.94±0.11
Drought	NIL	0.38±0.01	0.37±0.04	0.36±0.04	2.03±0.13	2.53±0.10	1.53±0.11	1.20±0.16
	NH <sub>4</sub> NO <sub>3</sub>	0.26±0.03	0.42±0.03	0.40±0.04	1.98±0.25	2.18±0.35	1.53±0.20	1.13±0.16
	Urine	0.35±0.04	0.44±0.05	0.42±0.05	1.98±0.25	1.89±0.21	1.05±0.11	1.02±0.13
Average by depth		0.36±0.01	0.42±0.01	0.37±0.01	1.89±0.08	1.93±0.08	1.21±0.07	1.03±0.05

$P<0.001$  for site×depth). The oxidation rates of sieved soil were approximately four times lower at *Früebüel* than at *Alp Weissenstein*.

The drought treatment had only little effect on CH<sub>4</sub> oxidation when soils were sieved and adjusted to identical water contents. At *Früebüel*, CH<sub>4</sub> oxidation in 0–5 cm soil depth slightly decreased under drought. At both sites, there was a tendency towards increased CH<sub>4</sub> oxidation in 10–15 cm soil depth.

N fertilization inhibited CH<sub>4</sub> oxidation of sieved soils, but effects were small and only present in the top soil layers. At *Früebüel*, fertilization reduced CH<sub>4</sub> oxidation in 0–5 cm soil depth with NH<sub>4</sub>NO<sub>3</sub> causing a stronger reduction (–25%) than urine (–10%). At *Alp Weissenstein*, NH<sub>4</sub>NO<sub>3</sub> reduced CH<sub>4</sub> oxidation in 0–5 cm soil depth, whereas urine caused a reduction in all soil depths.

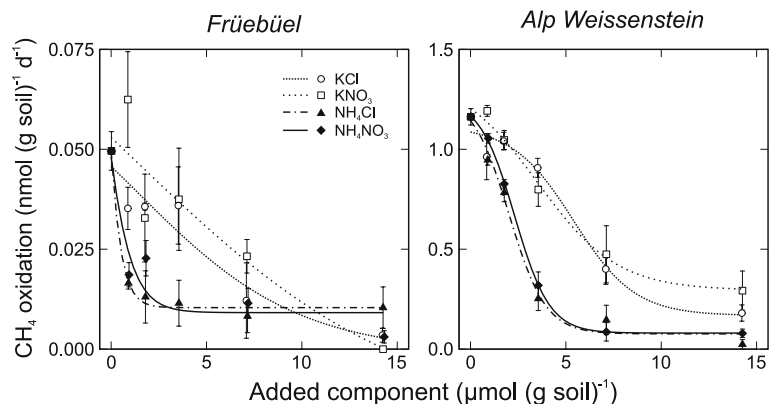
The addition of salts containing NH<sub>4</sub><sup>+</sup> inhibited CH<sub>4</sub> oxidation in sieved soil (Fig. 4). The addition of NH<sub>4</sub><sup>+</sup>-free salts also inhibited CH<sub>4</sub> oxidation, but only when

added at much higher concentrations ( $P<0.001$ ). NO<sub>3</sub><sup>-</sup>, K<sup>+</sup> and Cl<sup>-</sup> did not obviously differ in their effect.

## Discussion

Our study indicates that soil water content is the dominant factor regulating the temporal dynamics of soil CH<sub>4</sub> uptake, at least in well-aerated loamy-textured soils. At the whole-ecosystem level, low soil moisture resulted in a larger soil CH<sub>4</sub> sink, even under extreme drought. The experimental treatments applied had contrasting effects: The simulated summer drought of a few weeks had effects that lasted over the entire year and substantially altered the ecosystem's CH<sub>4</sub> balance; in contrast, even high N fertilizer application rates exerted only a transient inhibition of the ecosystem's CH<sub>4</sub> sink, suggesting that N inputs by grazing animals have only a rather small influence on soil CH<sub>4</sub> oxidation, at least in the pastures investigated.

**Fig. 4** CH<sub>4</sub> oxidation of sieved soil amended with different amounts of KCl, KNO<sub>3</sub>, NH<sub>4</sub>Cl or NH<sub>4</sub>NO<sub>3</sub>. The measured rates are expressed per soil dry weight. Lines are sigmoidal functions with intercept fitted for each compound (see Materials and Methods). Error bars indicate the standard errors of treatment means





## Effects of soil moisture

The grasslands investigated continuously acted as net sinks for atmospheric CH<sub>4</sub>. We never detected CH<sub>4</sub> emissions from soils, even when soils were water-logged after heavy rain or snow melt. Together with the fact that soil CH<sub>4</sub> concentrations always decreased with depth, this suggests the absence of substantial methanogenesis in deeper soil layers; however, we cannot exclude the possibility of methanogenesis in anaerobic micro-sites.

Soil moisture was the single most important factor controlling soil CH<sub>4</sub> uptake, explaining 65–85% of the temporal within-site variation in CH<sub>4</sub> fluxes. The reductions in soil moisture also fully explained the increased ecosystem-level CH<sub>4</sub> sink under simulated drought. Strong negative correlations of soil moisture and soil CH<sub>4</sub> uptake have also been reported from other studies (e.g. Castro et al. 1994; Price et al. 2004; Smith et al. 2000). This effect can easily be understood given the much slower diffusion of CH<sub>4</sub> and O<sub>2</sub> in water than in air (Kruse et al. 1996) and the general substrate limitation of soil CH<sub>4</sub> oxidation (Degelmann et al. 2009). If soil internal methanogenesis occurred at all, it likely was more important in moister soils and thus contributed to the correlation we observed. Both investigated sites were of loamy soil texture. It may well be that soil moisture control of CH<sub>4</sub> uptake is most pronounced in this texture class. Pore water retention may be too low in sandy soils and too high in clayey soils to effectively modulate diffusive constraints over time. Across texture classes, however, soil texture is a good predictor of soil CH<sub>4</sub> uptake (Dörr et al. 1993).

Many laboratory studies have shown an optimum soil water content beyond which diffusion limitation reduced CH<sub>4</sub> oxidation and below which physiological stress limited the activity of methanotrophs (Gulledge and Schimel 1998; Del Grosso et al. 2000) and such effects were also reported from field studies (Borken et al. 2006; Davidson et al. 2008; Fiedler et al. 2008; Dobbie and Smith 1996). In our study, no indications for reductions in ecosystem-level CH<sub>4</sub> uptake under water deficiency were found, not even during severe summer droughts. One possible explanation could be that drought stress can physiologically limit CH<sub>4</sub> oxidation by methanotrophs in the top soil but that this does not necessarily reduce the soil CH<sub>4</sub> uptake rates because increased CH<sub>4</sub> oxida-

tions in deeper, less dry soil layers can compensate for this effect.

Interestingly, the few weeks of simulated summer drought affected the soil's water balance throughout the entire year. Once the rain exclusion roofs had been removed, the recovery of soil moisture to ambient levels took several weeks to months and required multiple rain events. While the build-up of a soil moisture difference under the rain exclusion roofs can easily be explained by a reduction in precipitation, the mechanisms underlying the resilience after drought are less clear. Precipitation probably resulted in more runoff and deep seepage to ground water when the control plots were close to field capacity. However, there are also mechanisms which would tend to increase the soil moisture difference between the drought-treated and control plots and thus prolong the effect of drought. Drought induces a shrinking of soil aggregates, especially in soils with high clay content, and this often results in increased crack formation (Bronswijk 1988). This can lead to increased drainage after precipitation and a more efficient drying of soils due to a better coupling to the atmosphere (Ritchie and Adams 1974). In hydrophobic soils, water repellency often increases under drought (Doerr and Thomas 2000), which can substantially extend the time and amount of precipitation needed until a soil is re-saturated. Plants also affect evapotranspiration, but their role is less clear in the present study.

An intriguing effect found in our study was that effects of simulated summer drought on top soil moisture vanished in the winter following the treatment, but were found again in the next Spring and caused increased soil CH<sub>4</sub> uptake. This phenomenon was unexpected, since soils were covered by substantial amounts of snow during winter and the top soils of both control and drought-treated plots were water-saturated during snow-melt. We first suspected that the soils that had experienced simulated drought had developed hydrophobic properties; however, corresponding tests did not support this hypothesis (water droplet penetration time was less than 1 s in all samples; Dekker and Ritsema 1994). While vegetation can affect the soil's water balance, such an effect is rather unlikely since plant biomass did not differ at this time of the year (Hartmann et al., unpublished). We believe that the most likely explanation is that the top soil was re-saturated over winter but that deeper soil layers still exhibited water deficits relative to

control plots. Drainage and capillary forces may then have led to an accelerated drying of the top soil of formerly drought-exposed plots. This appears possible given the large amount of precipitation excluded, exceeding winter precipitation at both sites. However, no moisture data is available for deeper soil layers, so that no complete soil water balance can be set up and this mechanism therefore remains speculation. Irrespective of the mechanisms involved, this experiment demonstrated that relatively short episodes of severe drought can affect the soil  $\text{CH}_4$  balance for periods of up to 1 year.

We did not continuously measure  $\text{CH}_4$  fluxes at our field sites. We calculated a rough estimate of the cumulative effect of simulated drought on soil  $\text{CH}_4$  uptake by modeling  $\text{CH}_4$  fluxes in dependence of soil moisture. At *Früebühl*, simulated drought caused a 50% increase in the amount of  $\text{CH}_4$  oxidized in the course of the vegetation period ( $\sim 4.5$  and  $\sim 6.8$  mmol  $\text{CH}_4 \text{ m}^{-2}$  from April to October of 2007 and 2008 for control and drought-treatment, respectively; this corresponds to average soil uptake rates of  $\sim 21$  and  $32 \mu\text{mol CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ , respectively). We were not able to run the same calculation for *Alp Weissenstein* due to incomplete soil moisture records; however, the available data suggests that the relative increase was in the same range.

#### Effects of N fertilization

The application of  $\text{NH}_4\text{NO}_3$  and urine reduced soil  $\text{CH}_4$  uptake in our study, but inhibitory effects were rather small and recovery of the soil  $\text{CH}_4$  sink occurred within weeks, despite repeated applications of large amounts of N. These results are in line with other experiments testing effects of urine or  $\text{NH}_4\text{NO}_3$  application to pasture soils which reported strong, but transient inhibitory effects that vanished within days to weeks (cattle urine: Li and Kelliher 2007; Liebig et al. 2008;  $\text{NH}_4\text{NO}_3$ : Steinkamp et al. 2001). In contrast to these findings, several other field and laboratory studies have reported larger and long-lasting inhibitory effects of N application. However, these studies were conducted in ecosystems that did not regularly experience high mineral N applications, either from grazing animals or from synthetic fertilizers (e.g. Hütsch et al. 1993; Bronson and Mosier 1994).

How can this discrepancy be resolved? One possibility is that decades of grazing already have

resulted in reduced soil  $\text{CH}_4$  uptake by eliminating methanotrophs from soil micro-sites that are strongly affected by high  $\text{NH}_4^+$  concentrations, or by selecting for species that can tolerate high  $\text{NH}_4^+$  concentrations. In this case, the application of extra N would have had only little effect because the system was already constrained. Also, the effect of  $\text{NH}_4^+$  application strongly interacts with soil acidity (Hütsch et al. 1993, 1994), in part possibly because nitrification is reduced in acidic soils and more  $\text{NH}_4^+$  can therefore accumulate. Cattle urine generally is alkaline (Haynes and Williams 1992), thus locally increasing soil pH in acidic soils and thus possibly protecting methanotrophic micro-organisms from adverse effects of  $\text{NH}_4^+$ .

At the end of our study, 1 year after the last fertilizer application, N-effects on  $\text{CH}_4$  fluxes were detectable in the laboratory incubations of sieved soils but not in the soil  $\text{CH}_4$  uptake rates measured in situ. The effects we found in sieved soil decreased with depth, possibly indicating a shift of the active methanotrophic zone towards deeper soil layers. One possible explanation for this discrepancy may be that a fertilizer-induced reduction in top-soil  $\text{CH}_4$  oxidation was compensated by increased  $\text{CH}_4$  uptake in deeper layers.

Several studies have suggested that soil  $\text{CH}_4$  oxidation was not just inhibited by  $\text{NH}_4^+$  but also by a general “salt effect” of the fertilizer additions (Nesbit and Breitenbeck 1992; Gullledge and Schimel 1998; Price et al. 2004). Our laboratory investigations however do not support this view. While inhibitory effects of  $\text{NH}_4^+$ -free salts were found, these were much weaker than when  $\text{NH}_4^+$  was present. King and Schnell (1998) suggested that the inhibitory effect of  $\text{NH}_4^+$ -free salts may in fact be due to the desorption of  $\text{NH}_4^+$  from mineral surfaces by ion exchange processes, i.e. be  $\text{NH}_4^+$ -effects in disguise. However, Gullledge and Schimel (1998) have argued that the observed inhibitory effects of non-ammoniacal salts cannot be attributed to desorbed  $\text{NH}_4^+$  since the amounts of exchangeable  $\text{NH}_4^+$  were too low. In our study, the amount of  $\text{NH}_4^+$  potentially available for desorption was between 1.5 and  $2.0 \mu\text{mol (g soil)}^{-1}$  (soil extractions with 0.5 M KCl; Hartmann et al., unpublished). While the concentrations of ions added in these laboratory trials were small, we cannot exclude the possibility that desorbed  $\text{NH}_4^+$  may have contributed to the effects observed.

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## References

- Bodelier PLE, Laanbroek HJ (2004) Nitrogen as a regulatory factor of methane oxidation in soils and sediments. *FEMS Microbiol Ecol* 47:265–277
- Bodelier PLE, Roslev P, Henckel T, Frenzel P (2000) Stimulation by ammonium-based fertilizers of methane oxidation in soil around rice roots. *Nature* 403:421–424
- Borken W, Davidson EA, Savage K, Sundquist ET, Steudler P (2006) Effect of summer throughfall exclusion, summer drought, and winter snow cover on methane fluxes in a temperate forest soil. *Soil Biol Biochem* 38:1385–1395
- Bronson KF, Mosier AR (1994) Suppression of methane oxidation in aerobic soil by nitrogen fertilizers, nitrification inhibitors, and urease inhibitors. *Biol Fertil Soils* 17:263–268
- Bronswijk JJB (1988) Modeling of water-balance, cracking and subsidence of clay soils. *J Hydrol* 97:199–212
- Castro MS, Melillo JM, Steudler PA, Chapman JW (1994) Soil moisture as a predictor of methane uptake by temperate forest soils. *Can J For Res* 24:1805–1810
- Conrad R (2009) The global methane cycle: recent advances in understanding the microbial processes involved. *Env Microbiol Rep* 1:285–292
- Davidson EA, Nepstad DC, Ishida FY, Brando PM (2008) Effects of an experimental drought and recovery on soil emissions of carbon dioxide, methane, nitrous oxide, and nitric oxide in a moist tropical forest. *Glob Change Biol* 14:2582–2590
- Degelmann DM, Borken W, Kolb S (2009) Methane oxidation kinetics differ in European beech and Norway spruce soils. *Eur J Soil Sci* 60:499–506
- Dekker LW, Ritsema CJ (1994) How water moves in a water repellent sandy soil I. Potential and actual water repellency. *Water Resour Res* 30:2507–2517
- Del Grosso SJ, Parton WJ, Mosier AR, Ojima DS, Potter CS, Borken W, Brumme R, Butterbach-Bahl K, Crill PM, Dobbie K, Smith KA (2000) General CH<sub>4</sub> oxidation model and comparisons of CH<sub>4</sub> oxidation in natural and managed systems. *Glob Biogeochem Cycles* 14:999–1029
- Dobbie KE, Smith KA (1996) Comparison of CH<sub>4</sub> oxidation rates in woodland, arable and set aside soils. *Soil Biol Biochem* 28:1357–1365
- Doerr SH, Thomas AD (2000) The role of soil moisture in controlling water repellency: new evidence from forest soils in Portugal. *J Hydrol* 231:134–147
- Dörr H, Katruff L, Levin I (1993) Soil texture parameterization of the methane uptake in aerated soils. *Chemosphere* 26:697–713
- Dunfield PF (2007) The soil methane sink. In: Reay D, Hewitt CN, Smith K, Grace J (eds) *Greenhouse gas sinks*. CAB International, Wallingford, pp 152–170
- Dunfield P, Knowles R (1995) Kinetics of inhibition of methane oxidation by nitrate, nitrite, and ammonium in a humisol. *Appl Environ Microbiol* 61:3129–3135
- Fiedler S, Lamers M, Ingwersen J, Streck T, Stahr K, Jungkunst HF (2008) Impact of the heatwave in 2003 on the summer CH<sub>4</sub> budget of a spruce forest with large variation in soil drainage: a four-year comparison (2001–2004). *J Plant Nutr Soil Sci* 171:666–671
- Gilgen AK, Buchmann N (2009) Response of temperate grasslands at different altitudes to simulated summer drought differed but scaled with annual precipitation. *Biogeosci* 6:2525–2539
- Gulledge J, Schimel JP (1998) Low-concentration kinetics of atmospheric CH<sub>4</sub> oxidation in soil and mechanism of NH<sub>4</sub><sup>+</sup> inhibition. *Appl Environ Microbiol* 64:4291–4298
- Gulledge J, Doyle AP, Schimel JP (1997) Different NH<sub>4</sub><sup>+</sup>-inhibition patterns of soil CH<sub>4</sub> consumption: a result of distinct CH<sub>4</sub>-oxidizer populations across sites? *Soil Biol Biochem* 29:13–21
- Haynes RJ, Williams PH (1992) Changes in soil solution composition and pH in urine affected areas of pasture. *J Soil Sci* 43:323–334
- Haynes RJ, Williams PH (1993) Nutrient cycling and soil fertility in the grazed pasture ecosystem. *Adv Agron* 49:119–199
- Hütsch BW, Webster CP, Powlson DS (1993) Long-term effects of nitrogen-fertilization on methane oxidation in soil of the Broadbalk Wheat Experiment. *Soil Biol Biochem* 25:1307–1315
- Hütsch BW, Webster CP, Powlson DS (1994) Methane oxidation in soil as affected by land-use, soil pH and N fertilization. *Soil Biol Biochem* 26:1613–1622
- IPCC (2007) *Climate change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge
- King GM, Schnell S (1998) Effects of ammonium and non-ammonium salt additions on methane oxidation by *Methylosinus trichosporium* OB3b and Maine forest soils. *Appl Environ Microbiol* 64:253–257
- Kruse CW, Moldrup P, Iversen N (1996) Modeling diffusion and reaction in soils: II. Atmospheric methane diffusion and consumption in a forest soil. *Soil Sci* 161:355–365
- Le Mer J, Roger P (2001) Production, oxidation, emission and consumption of methane by soils: a review. *Eur J Soil Biol* 37:25–50
- Lessard R, Rochette P, Gregorich EG, Desjardins RL, Pattey E (1997) CH<sub>4</sub> fluxes from a soil amended with dairy cattle manure and ammonium. *Can J Soil Sci* 77:179–186

- Li Z, Kelliher FM (2007) Methane oxidation in freely and poorly drained grassland soils and effects of cattle urine application. *J Environ Qual* 36:1241–1248
- Liebig MA, Kronberg SL, Gross JR (2008) Effects of normal and altered cattle urine on short-term greenhouse gas flux from mixed-grass prairie in the Northern Great plains. *Agric Ecosyst Environ* 125:57–64
- Mosier A, Schimel D, Valentine D, Bronson K, Parton W (1991) Methane and nitrous oxide fluxes in nature, fertilized and cultivated grasslands. *Nature* 350:330–332
- Nesbit SP, Breitenbeck GA (1992) A laboratory study of factors influencing methane uptake by soils. *Agric Ecosyst Environ* 41:39–54
- Powlson DS, Goulding KWT, Willison TW, Webster CP, Hütsch BW (1997) The effect of agriculture on methane oxidation in soil. *Nutr Cycl Agroecosyst* 49:59–70
- Price SJ, Sherlock RR, Kelliher FM, McSeveny TM, Tate KR, Condron LM (2004) Pristine New Zealand forest soil is a strong methane sink. *Glob Change Biol* 10:16–26
- R Development Core Team (2010) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Ritchie JT, Adams JE (1974) Field measurements of evapotranspiration from soil shrinkage cracks. *Soil Sci Soc Am J* 38:131–134
- Smith KA, Dobbie KE, Ball BC, Bakken LR, Sitaula BK, Hansen S, Brumme R, Borken W, Christensen S, Prieme A, Fowler D, Macdonald JA, Skiba U, Klemmedtsson L, Kasimir-Klemmedtsson A, Degorska A, Orlanski P (2000) Oxidation of atmospheric methane in Northern European soils, comparison with other ecosystems, and uncertainties in the global terrestrial sink. *Glob Change Biol* 6:791–803
- Steinkamp R, Butterbach-Bahl K, Papen H (2001) Methane oxidation by soils of an N limited and N fertilized spruce forest in the Black Forest, Germany. *Soil Biol Biochem* 33:145–153
- Willison TW, Webster CP, Goulding KWT, Powlson DS (1995) Methane oxidation in temperate soils: effects of land use and the chemical form of nitrogen fertilizer. *Chemosphere* 30:539–546
- Zeeman MJ, Hiller R, Gilgen AK, Michna P, Plüss P, Buchmann N, Eugster W (2010) Management and climate impacts on net CO<sub>2</sub> fluxes and carbon budgets of three grasslands along an elevational gradient in Switzerland. *Agric For Meteorol* 150:519–530